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Fusarium species of the *Gibberella fujikuroi* complex and fumonisin contamination of pearl millet and corn in Georgia, USA

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Abstract

This study was designed to identify and compare the *Fusarium* species of the *Gibberella fujikuroi* complex on pearl millet (*Pennisetum glaucum* (L.) R. Br) and corn (*Zea mays* L.) crops grown in southern Georgia, and to determine their influence on potential fumonisin production. Pearl millet and corn samples were collected in Georgia in 1996, 1997 and 1998. Three percent of the pearl millet seeds had fungi similar to the *Fusarium* species of the *G. fujikuroi* species complex. One hundred and nineteen representative isolates visually similar to the *G. fujikuroi* species complex from pearl millet were paired with mating population A (*Fusarium verticillioides* (Sacc.) Nirenberg), mating population D (*F. proliferatum* (Matsushima) Nirenberg) and mating population F (*F. thapsinum* (Klittich, Leslie, Nelson and Marasas) tester strains. Successful crosses were obtained with 50.4%, 10.1% and 0.0% of these isolates with the A, D and F tester strains, while 39.5% of the isolates did not form perithecia with any tester strains. Two of the typical infertile isolates were characterized by DNA sequence comparisons and were identified as *Fusarium pseudonygamai* (Nirenberg and O'Donnell), which is the first known isolation of this species in the United States. Based on the pattern of cross-compatibility, conidiogenesis, colony characteristics and media pigmentation, a majority of the infertile isolates belong to this species. Fumonisins FB_1 and FB_2 were not detected in any of the 81 pearl millet samples analyzed. The species of the *G. fujikuroi* species complex were dominant in corn and were isolated from 84%, 74% and 65% of the seed in 1996, 1997 and 1998, respectively. Representative species of the *G. fujikuroi* species complex were isolated from 1996 to 1998 Georgia corn survey (162, 104 and 111 isolates, respectively) and tested for mating compatibility. The incidence of isolates belonging to mating population A (*F. verticillioides*) ranged from 70.2% to 89.5%. Corn survey samples were assayed for fumonisins, and 63% to 91% of the 1996, 1997 and 1998 samples were contaminated. The total amount of fumonisins in the corn samples ranged from 0.6 to 33.3 μg/g.

Key words: corn, fumonisins, *Fusarium, G. fujikuroi,* mating population, pearl millet, *Pennisetum glaucum*, *Zea mays*

Introduction

Fungi belonging to the *Gibberella fujikuroi* (Sawada) Wollenw. species complex have a wide host range on a variety of economically important plants including corn and sorghum [1]. This species complex includes all members of the *Fusarium* section Liseola *sensu* Nelson et al. [2] and about four dozen other species [3–5]. Members of this group can cause disease during all developmental stages of the plant. *Fusarium verticillioides* and *F. proliferatum* often infect corn through wounds caused by insects such as the European corn borer *Ostrinia nubilalis* [6, 7]. *F. verticillioides* is the main fungal species producing fumonisin mycotoxins in North America [8]. The naturally occurring fumonisins, especially fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2), are detected at biologically

significant levels in corn and corn-based human foodstuffs and animal feeds in several countries worldwide [9–12]. Fumonisins can cause fatal leukoencephalomalacia in horses, pulmonary edema in swine, and cancer in laboratory male rats and female mice [13, 14].

Pearl millet is widely cultivated as a staple grain for human consumption throughout the tropical and subtropical regions of the world [15]. It is being developed as a promising alternative feed grain crop for low-input agricultural production systems in the southeastern United States. Ten *Fusarium* species have been isolated from pearl millet grain in several African countries [16]. The objective of this study was to identify and compare the *Fusarium* species of the *G. fujikuroi* species complex from pearl millet and corn crops grown in southwest Georgia.

Materials and methods

Pearl millet (hybrid HGM 100) was grown in Plains, Georgia (Sumter Co.) annually from 1996 to 1998. Four replications were grown in several rotations. After combine harvesting grain was dried, mixed together and kept in storage (4 °C) separately by year. Kernels were plated on potato-dextrose agar (Difco, Sparks, MI) at 10 kernels per plate (100 × 15 mm). Plates were incubated at 25 °C and examined after 5 days. One hundred and nineteen representative isolates (41, 35, 43 isolates from 1996, 1997 and 1998 samples, respectively) of fungi in the *G. fujikuroi* complex were paired with mating population A (*F. verticillioides*) FGSC # 7598 (A+ tester strain) and FGSC # 7600 (A−tester strain), mating population D (*F. proliferatum*) FGSC # 7614 (D+ tester strain) and FGSC #7615 (D− tester strain) and mating population F (*F. thapsinum*) FGSC # 7055 (F+ tester strain) and FGSC # 7054 (F− tester strain) tester strains. All tester strains were obtained from the Fungal Genetic Stock Center at the University of Kansas Medical Center, Kansas City, KS. Crosses were carried out on V-8 juice agar with a modification of the method of Klittich and Leslie [17, 18]. Testers were grown on potato-dextrose broth with shaking (wrist action shaker) and incubated at 25 °C on a 12 h dark/light cycle. Two 3-ml aliquots of spore suspensions were added to 10-day-old cultures of the field isolates grown on V-8 juice agar. The spore suspension of the tester isolates was distributed on the surface of the field isolates by a sterile glass rod. Examinations for the development of perithecia were carried out by eye and under a dissecting microscope. Mated cultures were considered inter-fertile if perithecia formed by 35 days.

Two isolates from pearl millet with the characteristic growth, morphology and pigmentation of the isolates that did not form perithecia in mating analyses were evaluated by DNA sequence comparisons. These isolates have been deposited in the Fusarium Research Center culture collection, under accession numbers M-8722 and M-8723. Isolates were grown on carnation leaf agar for 7–10 days until the carnation leaves were covered with mycelium and sporodochia. DNA extractions were performed on infested carnation leaves using a DNEasy Plant DNA miniprep kit (QIAgen, Valencia, CA), using the manufacturer's instructions. A portion of the translation elongation factor 1-alpha (tef) gene was amplified from 1 μl of genomic DNA using the polymerase chain reaction (PCR) and sequenced on an ABI 377 automated DNA sequencer, as described previously [19]. The sequences produced were assembled using Sequencher v.3.0 software (GeneCodes, Inc., Ann Arbor, MI) and edited for accuracy. Searches for identity were performed against the GenBank database by using BLAST at the National Center for Biotechnology website (http://www.ncbi.nih.gov/BLAST/) and against a local database of representative *Fusarium* tef DNA sequences (K. O'Donnell, personal communication). Sequences were added manually to an existing alignment of sequences from the *G. fujikuroi* species complex [5]. Maximum parsimony analysis was performed using the software package PAUP v.4.0b9 [20]. Heuristic searches employing TBR branch-swapping and random sequence addition (10 replicates) were performed. Gaps were coded as missing characters, with the exception of 13 gap positions which were encoded as a fifth character state. Bootstrap analyses were performed in 1000 replicates as described above, except that simple sequence addition was used.

Preharvest corn samples were randomly collected in the field from 41 to 43 counties in Georgia from 1996 to 1998, dried to 10% moisture and stored at 4 °C until analysis. Kernels were plated on Dichloran-Glycerol (DG18) medium

[21] at 10 kernels per plate. Plates were incubated at 28 °C and examined after 7–10 days. Three hundred and seventy seven *Fusarium* isolates representative of the *G. fujikuroi* species complex were obtained from the 1996, 1997 and 1998 surveys (2–4 isolates per county per year with 162, 104 and 111 isolates, respectively) and tested for mating population. Crosses were carried out as previously described.

Analysis of fumonisins was performed according to the procedure of Visconti and Doko [22] as modified by Visconti and Pascale [23]. Details of this method are in Jurjevic et al. [12]. Pearl millet and corn samples were spiked with 10 ppm of fumonisin B_1 and 5 ppm of fumonisin B_2. The recoveries were somewhat low with 58–67% recovery for the pearl millet samples and 79–82% recovery for the corn samples. The detection limit

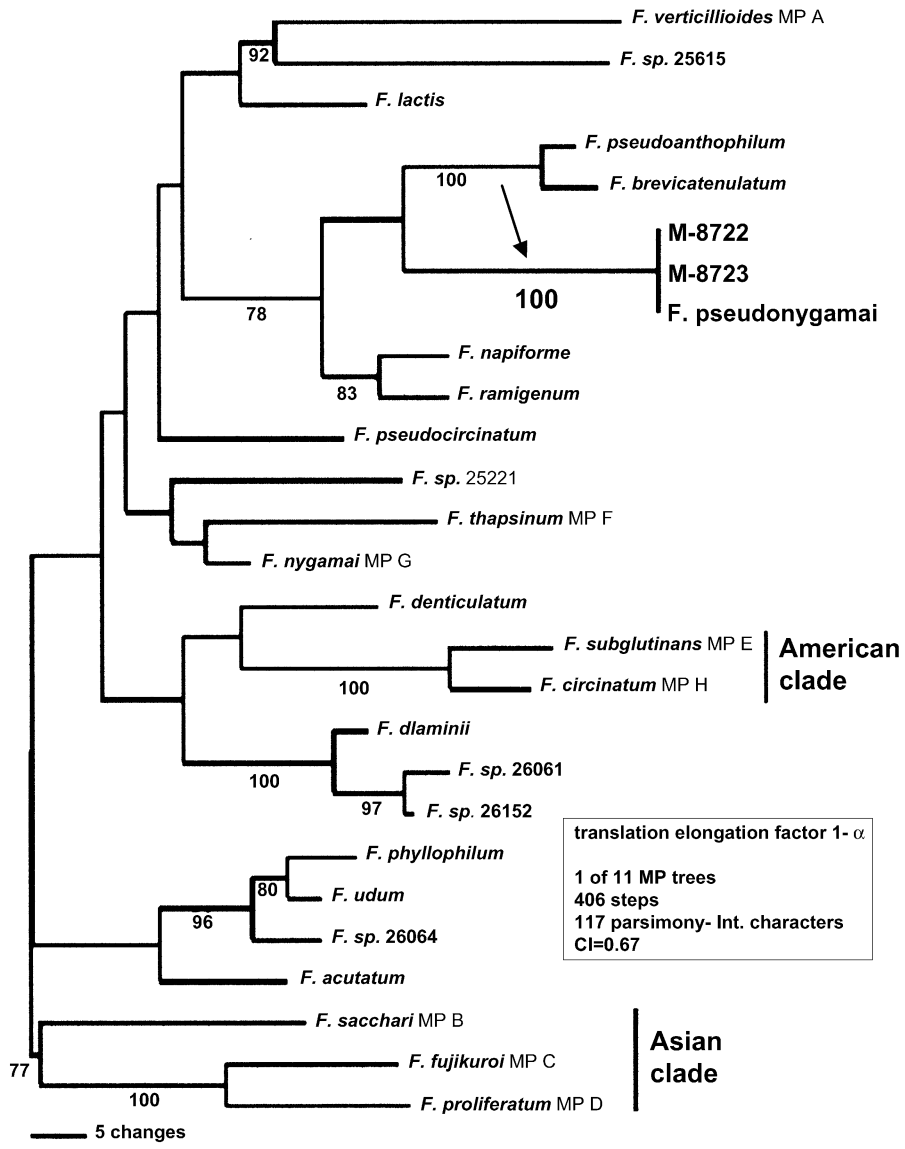

Figure 1. One of 11 most parsimonious trees showing the relationship of pearl millet isolates M-8722 and M-8723 to members of the *Gibberella fujikuroi* species complex. The so called 'African' clade corresponds to all species not listed as 'American' or 'Asian', and the elongation factor 1-alpha gene does not show it to be monophyletic. Bootstrap values based on 1000 replicates are shown below branches.

was about 0.1 ppm for pearl millet and 0.05 ppm for corn.

Results and discussion

Fungi of the *G. fujikuroi* species complex were not dominant in pearl millet. The most commonly isolated fungi in this study included: *F. semitectum* (up to 31%), *F. chlamydosporum* (up to 31%) *Alternaria* spp. (up to 29%), *Curvularia* spp. (up to 28%), and *Aspergillus flavus* (Link), (up to 14%), which is similar to that reported by Wilson et al. [24]. The major difference in this study and the one by Wilson et al. [24] was that *A. flavus* was common in 1996 and 1998. Only 3% of the pearl millet seeds had fungi similar to species from the *G. fujikuroi* species complex. One hundred and nineteen representative isolates of the *G. fujikuroi* complex were obtained from pearl millet and were paired with mating population A (*F. verticillioides*), D (*F. proliferatum*) and F (*F. thapsinum*) tester strains. No successful crosses were obtained with mating population F, 50.4% (60 of 119) of the isolates from pearl millet were fertile with mating population A and 10.1% (12 of 119) crossed with mating population D. A high percentage (39.5%) or 47 isolates of 119 did not form any perithecia in controlled matings.

To determine the species of the infertile isolates partial sequences of the translation elongation factor 1-alpha (tef) gene were determined for two representative isolates M-8722 and M-8723. Those isolates had morphological and pigmentation characteristics common among the infertile isolates. BLAST searches comparing these sequences with those in the GenBank database both showed 100% identity with *Fusarium pseudonygamai* Nirenberg and O'Donnell isolate NRRL 13592 [4], a member of the African clade of the *G. fujikuroi* species complex and known only from pearl millet grown in Nigeria (Figure 1). Microscopic analysis of these isolates showed that they possessed typical cultural and morphological characteristics of this species.

Fumonisins FB_1 and FB_2 were not detected in any of the 81 analyzed pearl millet samples.

Fusarium species of the *G. fujikuroi* complex were dominant in the corn samples from 1996, 1997 and 1998 (84.2%, 78%. 65.1%, respectively). Other *Fusarium* species were rare. Of the 377 representative isolates evaluated, the incidence of isolates belonging to mating population A (*F. verticillioides*) ranged from 70.2% to 89.5%. In the 1996, 1997 and 1998 samples, 86%, 63% and 91% of samples respectively were contaminated with fumonisins and concentrations ranged from 0.6 to 33.3 μg/g (Figure 2).

Pearl millet has been grown for many years in southern Georgia as an experimental but promising alternative feed grain for low-input production systems in the southeastern United States. In a recent study, the dominant *Fusarium* species found on pearl millet were *F. semitectum* (Berk. & Rav.) and *F. chlamydosporum* (Wollenw. & Reinking) [25]. Only 3% of the pearl millet seeds in this study had fungi similar to species of the *G. fujikuroi* species complex. Among these fungi, *Fusarium pseudonygamai* was isolated from pearl millet for the first time in the United States. The possibility that these infertile isolates belonged to other *Fusarium* species was eliminated by the mating compatibility studies, colony morphology and by media pigmentation. Isolates evaluated by DNA analysis (M-8722 and M-8723) were 100% typical with *F. pseudonygamai* of isolate NRRL 13592 [4] known only from pearl millet grown in Nigeria. Fumonisins FB_1 and FB_2 were not detected in any of the 81 analyzed grain samples. According to Fotso et al. [26], *F. pseudonygamai* does not produce fumonisins.

In contrast to the isolates from pearl millet, *F. verticillioides* was the most commonly isolated *Fusarium* species from corn harvested in southern Georgia. *Fusarium verticillioides* is a widely dis-

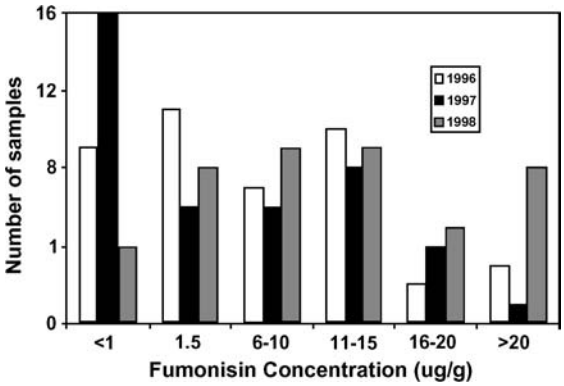

Figure 2. Distributions of fumonisin concentration in corn samples harvested in southern Georgia in 1996, 1997 and 1998. Samples were obtained from 42, 41 and 43 counties in each respective year.

tributed pathogen of corn and causes seedling diseases, root rots, stalk rots and ear or kernel rots, and can also occur in symptomless corn [7, 27]. *Fusarium* species that occurred in the samples from this study were isolated and identified following the classification system of Nelson et al. [2], and Gerlach and Nirenberg [28], and by using sequences from the tef gene. In this study, 70% to 90% of the isolates from corn belonging to the *G. fujikuroi* species complex (based on morphological and cultural characteristics) produced perithecia when combined with a compatible mating population A tester strain. This survey showed both a high incidence of contamination and a high concentration of fumonisins on corn grown in south Georgia. In contrast there seemed to be a low risk of fumonisin contamination of pearl millet. The most logical explanation for these differences is that the incidence *of Fusarium* species that may be fumonisin producers was low in pearl millet compared to corn.

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